

Scotland's Rural College

## **Pyrolysis gas-chromatography mass-spectrometry (Py-GC/MS) to identify compression wood in *Pinus radiata* saplings**

Brennan, M; McLean, JP; Klingberg, A; Altaner, C; Harris, PJ

*Published in:*  
Holzforschung

*DOI:*  
[10.1515/hf-2013-0110](https://doi.org/10.1515/hf-2013-0110)

Print publication: 01/01/2014

*Document Version*  
Publisher's PDF, also known as Version of record

[Link to publication](#)

### *Citation for published version (APA):*

Brennan, M., McLean, JP., Klingberg, A., Altaner, C., & Harris, PJ. (2014). Pyrolysis gas-chromatography mass-spectrometry (Py-GC/MS) to identify compression wood in *Pinus radiata* saplings. *Holzforschung*, 68(5), 505 - 517. <https://doi.org/10.1515/hf-2013-0110>

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Maree Brennan<sup>a</sup>, J. Paul McLean<sup>b</sup>, Andreas Klingberg<sup>c</sup>, Clemens Altaner<sup>d</sup> and Philip J. Harris\*

# Pyrolysis gas-chromatography mass-spectrometry (Py-GC/MS) to identify compression wood in *Pinus radiata* saplings

**Abstract:** The potential of pyrolysis followed by gas-chromatography and mass-spectrometry (Py-GC/MS) was investigated for identifying compression wood (CW) in saplings of radiata pine (*Pinus radiata*) by examining samples of CW and opposite wood (OW). Phenolic compounds and anhydrosugars were identified among the pyrolysis products that provided information about the cell-wall polymers. Sample preparation, such as coarse-milling, fine-milling, and fine-milling followed by calcium-chloride treatment was also investigated. Fine-milling typically decreased the total yield of phenolic compounds compared with coarse-milling. Fine-milling followed by calcium-chloride washing significantly increased the proportions of pyrolysis products from polysaccharides, specifically from (1→4)-β-D-galactans that were of interest in distinguishing CW from OW. Six pyrolysis products were identified that were unique to the CW samples examined, including derivatives of (1→4)-β-D-galactans and H-units of lignin. Other pyrolysis products were identified that had significantly different proportions between the two wood types, and sometimes among samples of the same wood type.

**Keywords:** (1→4)-β-galactan, compression wood detection, H-units of lignin, lignin, pyrolysis gas-chromatography mass-spectrometry (Py-GC/MS), radiata pine

<sup>a</sup>Present address: Crop and Soils Systems, Scotland's Rural College, King's Buildings, West Mains Road, Edinburgh, Scotland EH9 3JG.

<sup>b</sup>Present address: Forest Research, Northern Research Station, Roslin, Midlothian, Scotland EH25 9SY.

<sup>c</sup>Present address: Department of Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany.

<sup>d</sup>Present address: New Zealand School of Forestry, The University of Canterbury, Private Bag 4800, Christchurch, New Zealand.

\*Corresponding author: Philip J. Harris, School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand, Phone: +64-9-373-7599 ext. 88366, Fax: +64-9-373-7417, e-mail: p.harris@auckland.ac.nz

Maree Brennan, J. Paul McLean and Clemens Altaner: School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand

Andreas Klingberg: Department of Wood Science, University of Hamburg, Leuschnerstraße 91, 21031 Hamburg, Germany

## Introduction

Reaction wood is formed in trees in response to displacement of a stem or branch from its equilibrium growth position, for example when stems are displaced from the vertical (Scurfield 1973). In softwoods (coniferous gymnosperms), the reaction wood is on the underside of leaning stems and is called compression wood (CW); the wood formed on the opposite side of these stems is known as opposite wood (OW) (Timell 1986). CW is considered to be a defect in structural timber because of its inferior mechanical properties and higher longitudinal shrinkage than in the case of normal wood (NW) or OW, on drying. When CW and NW are present in one piece of timber, the heterogeneous shrinkage on drying will often result in distortion. CW is also less desirable for pulp and paper production due to its differing chemical and fibre properties. Thus, there is a need for detecting CW analytically.

Softwood cells are composed predominantly of tracheids with thick cell walls that are composed of cellulose microfibrils embedded in a matrix of lignin and non-cellulosic polysaccharides. In NW and OW, the lignin consists mostly of guaiacyl units (G-units), and the non-cellulosic polysaccharides are *O*-acetyl-galactoglucomannans (AcGGM), and 4-*O*-methylglucuronarabinoxylans (MeGluAX) (Whistler and Chen 1991; Harris 2005; Harris and Stone 2008). However, the walls of CW tracheids, which are morphologically different to NW tracheids (Butterfield and Meylan 1980; Donaldson et al. 2004), have a lower cellulose and a higher lignin content than walls of NW tracheids (Timell 1986). CW lignin contains *p*-hydroxyphenyl units (H-units), which are nearly absent in NW lignin (Bland 1958; Westermark 1985; Nanayakkara et al. 2009; Brennan et al. 2012). The walls of CW tracheids also contain smaller proportions of AcGGM, and MeGluAX, but greater proportions of (1→4)-β-D-galactans (up to 10%) than those of NW tracheids (Bouveng and Meier 1959; Timell 1986; Altaner et al. 2010; Brennan et al. 2012).

CW of *Pinus radiata* (radiata pine) can be distinguished from OW and NW by detailed chemical analysis of

the cell-wall polymers (Nanayakkara et al. 2009; Brennan et al. 2012). Pyrolysis followed by GC/MS (Py-GC/MS) is an established method of biopolymer analysis (Mohan et al. 2006; Torri et al. 2010). Py-GC/MS combined with principal component analysis (PCA) is a promising technique for identifying CW (Alves et al. 2009). Pyrolysis of lignin yields typical phenolic products, which may be substituted with one or two OMe groups depending on the lignin type (Ralph and Hatfield 1991; Möller et al. 2003; Rencoret et al. 2011; del Río et al. 2012), whereas polysaccharides yield anhydrosugars (Shafizadeh and Fu 1973; Ponder et al. 1990; Moldoveanu 1998) and other non-specific products.

Milling of wood (Ikeda et al. 2001) and pulps (Syverud et al. 2003) influences the yield of pyrolysis products but not their relative abundance to each other (Galletti and Bocchini 1995). The pyrolysis of coarser particles is supposed to be less isothermal at higher temperatures than at lower temperatures (Beaumont and Schwob 1984), but it is not clear whether particle size influences the ability to distinguish between different wood types.

Pyrolysis is catalysed by Na and K cations, which lead to non-specific degradation products with low molecular weights in the case of polysaccharides (Pan and Richards 1989; Kleen and Gellerstedt 1995). Ca cations, on the other hand, have no effect, thus an exchange of Na for Ca cations by washing samples with a solution of  $\text{CaCl}_2$  increases the anhydrosugar yields (Kleen and Gellerstedt 1995). It is likely that the yields of pyrolysis products of (1→4)- $\beta$ -D-galactans, as a significant component of CW, would also be increased by washing with  $\text{CaCl}_2$ .

The aim of this study was to investigate the potential of Py-GC/MS as a method of identifying the presence of CW in wood from saplings of *Pinus radiata*. To this end, the relative yields of the phenolic compounds and anhydrosugars obtained from CW and OW were compared as a function of the particle size before pyrolysis. Because of the importance of (1→4)- $\beta$ -D-galactans for CW identification, the effects of washing the samples with  $\text{CaCl}_2$  before pyrolysis was also examined.

## Materials and methods

### Wood samples

Wood samples were obtained from 18-month-old saplings of *Pinus radiata* grown in the field at Amberley, Canterbury, New Zealand. The trees had an average under-bark diameter of 33.7 mm, and were <3 m in height. The saplings were tilted at approx. 25° from the vertical

to produce pure CW and pure OW (Apiolaza et al. 2011a). The cross-sections of these wood types were characterised anatomically by Apiolaza et al. (2011b). At harvest, the stems were vertically split to obtain CW and OW samples, which were dried at 35°C to constant mass. CW was identified visually by wetting the wood samples, after which CW has a darker appearance than OW. Six samples of each wood type from different trees were chosen for pyrolysis. As a reference standard for (1→4)- $\beta$ -D-galactans (galactan), lupin seed galactan was pyrolysed; this galactan had been treated with  $\alpha$ -L-arabinofuranosidase to remove any attached arabinosyl residues (catalogue reference P-GALLU; Megazyme International Ireland Ltd., Bray, Ireland).

### Sample preparation for pyrolysis

1. *Coarse* samples were prepared by milling in a Wiley® mini-mill (Thomas Scientific, Swedesboro, NJ, USA) to pass a 40 mesh screen (422  $\mu\text{m}$  pore size). 2. *Fine* samples were prepared from ~50 mg of each of the coarse samples, which were cooled for 15 min and milled in liquid  $\text{N}_2$  in a SPEX CertiPrep 6750 Freezer/Mill (Spex, Metuchen, NJ, USA). Total milling time was 3 min (3 cycles of 1 min milling, with 2 min cooling intervals). 3. *Calcium-treated* samples were prepared by washing ~25 mg of the fine samples in 0.1 M  $\text{CaCl}_2$  (500  $\mu\text{l}$ , 30 min), followed by washing twice in double-distilled water (500  $\mu\text{l}$ , 10 min). All samples were dried in a vacuum oven (35°C) containing  $\text{P}_2\text{O}_5$  (18 h), and weighed (~65–85  $\mu\text{g}$ ) into a pyrolysis cup. Three replicate portions of each sample were analysed.

### Pyrolysis GC/MS at 500°C

Instrument: Py-2020iD micro-furnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan); interface temperature: 360°C. GC/MS instrument: Agilent 6890 GC with a FID detector and an Agilent 5973 mass selective detector (Agilent Technologies, Santa Clara, CA, USA), 70 eV, scan-range: 15–600 m/z; ion source temp.: 230°C. Split injection: 1:30; column: DB-1701 fused silica (60 m×0.25 mm; 0.25  $\mu\text{m}$ ) from Agilent; carrier gas: He (1 ml min<sup>-1</sup>); injection port temp.: 250°C; temp. programme: 4 min at 45°C followed by ramping at 3°C min<sup>-1</sup> to 280°C and held for 20 min; temp. of GC/MS interface and FID: 280°C. Samples were analysed in random order. Identification of the pyrolysis products was done at the Center of Wood Science and Technology, University of Hamburg, Germany, based on an in-house-library of mass spectra (Faix et al. 1990, 1991; Bremer 1991) and the NIST 02 library [National Institute of Standards and Technology (2002)]. Lupin seed (1→4)- $\beta$ -D-galactan was pyrolysed in the same way.

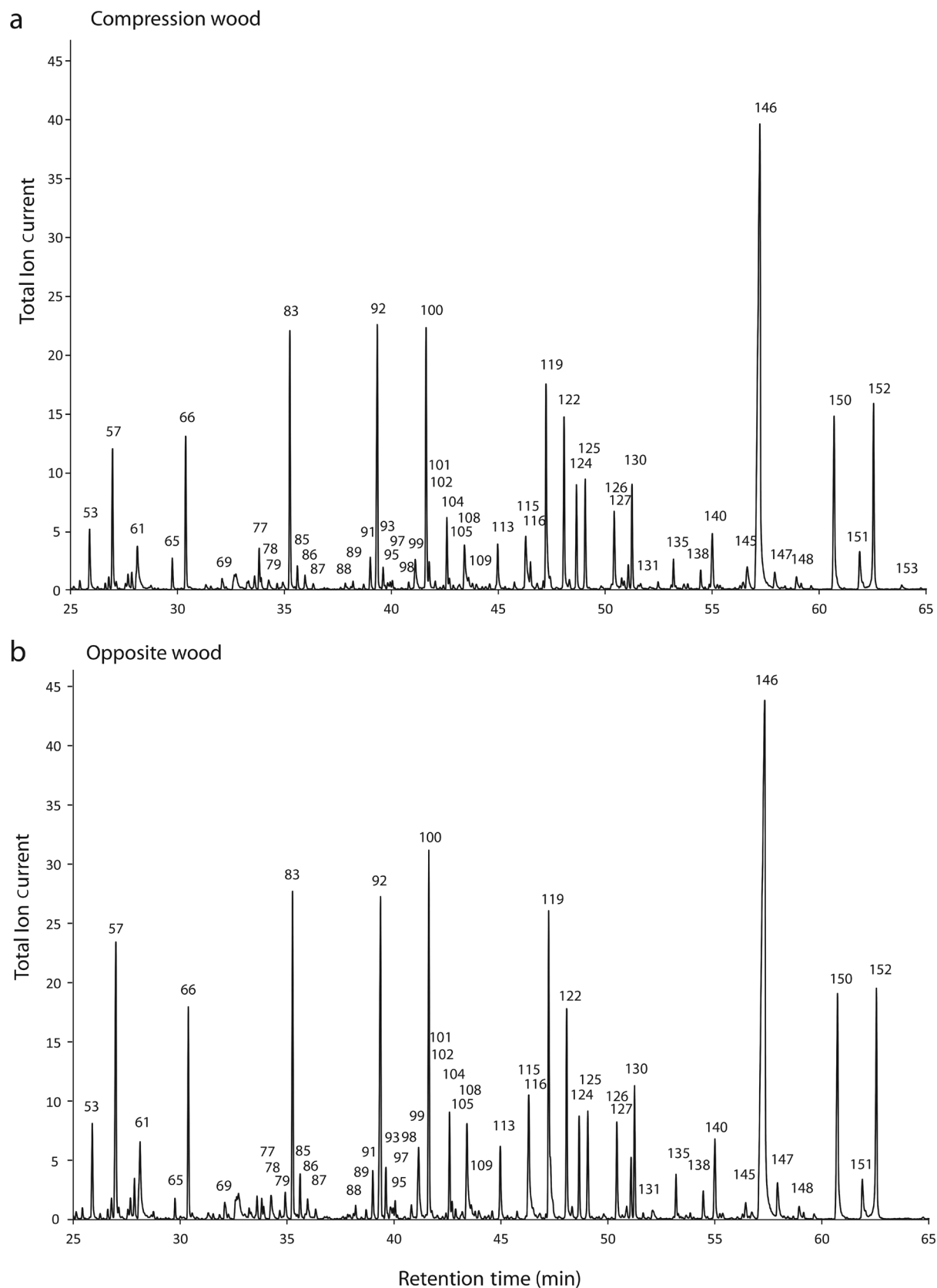
### Processing of chromatographic data

The total ion current (TIC) chromatograms were baseline corrected with *metAlign* software, version 080311 (Lommen 2009). Peak integration: *Wsearch32* Mass Spectrometry software, version 1.6.2005 (<http://www.wsearch.com.au>), with a minimum threshold for peak area of 0.05%. The data are presented as relative percentage of the normalised total peak area (=100%).

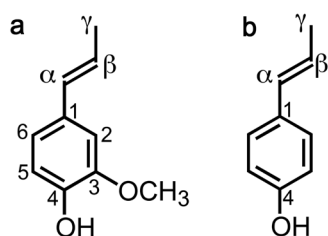
**Table 1** Pyrolysis products identified in the retention-time-range of interest in OW and CW samples.

| Peak <sup>a</sup> | RT <sup>b</sup> | Identification <sup>c</sup>                         | Origin <sup>d</sup> | Wood type | Base ion <sup>e</sup> (100) | Ion m/z (relative intensity)   |
|-------------------|-----------------|---|---------------------|-----------|-----------------------------|--|
| 65                | 29.75           | Phenol  | Ph                  | Both      | 94                          | 66 (27), 65 (21), 39 (13), 95 (7), 40 (6), 55 (6), 63 (5), 38 (4), 51 (4)              |
| 66                | 30.38           | Guaiacol [2-methoxyphenol]                          | G                   | Both      | 109                         | 124 (87), 81 (54), 53 (13), 52 (7), 110 (7), 125 (7), 51 (6), 39 (5), 65 (5)           |
| 69                | 32.09           | 2-Methylphenol                                      | Ph                  | Both      | 108                         | 107 (90), 79 (35), 77 (33), 90 (22), 89 (14), 80 (13), 51 (11), 53 (9), 39 (9)         |
| 77                | 33.81           | 4-Methylphenol                                      | Ph                  | Both      | 107                         | 108 (83), 77 (27), 123 (19), 79 (18), 138 (14), 51 (9), 90 (8), 53 (7), 80 (7)         |
| 78                | 33.90           | 3-Methylphenol                                      | Ph                  | Both      | 108                         | 107 (96), 79 (29), 77 (28), 53 (9), 90 (9), 51 (9), 80 (8), 39 (8), 109 (8)            |
| 83                | 35.25           | 4-Methylguaiacol                                    | G                   | Both      | 138                         | 123 (92), 95 (28), 67 (16), 77 (15), 55 (11), 139 (9), 65 (8), 39 (8), 51 (7)          |
| 86                | 35.96           | 3,4-Dimethylphenol                                  | Ph                  | Both      | 122                         | 107 (99), 121 (51), 56 (31), 77 (25), 91 (25), 79 (15), 51 (11), 26 (11), 108 (11)     |
| 88                | 37.84           | 4-Ethylphenol                                       | H                   | CW        | 107                         | 122 (32), 77 (15), 108 (8), 39 (5), 91 (4), 51 (4), 78 (4), 79 (3), 103 (3)            |
| 89                | 38.20           | 3-Ethylguaiacol                                     | G                   | Both      | 152                         | 137 (95), 109 (23), 79 (17), 91 (12), 77 (12), 151 (9), 107 (8), 51 (7), 108 (7)       |
| 91                | 39.01           | 4-Ethylguaiacol                                     | G                   | Both      | 137                         | 152 (40), 122 (11), 138 (9), 91 (9), 77 (7), 94 (6), 79 (5), 65 (5), 148 (4)           |
| 99                | 41.12           | (1,5)-Anhydroarabinofuranose                        | Ps                  | Both      | 57                          | 73 (53), 29 (30), 43 (25), 60 (22), 55 (19), 86 (17), 58 (17), 45 (15), 42 (15)        |
| 100               | 41.62           | 4-Ethylguaiacol                                     | G                   | Both      | 150                         | 135 (80), 107 (30), 77 (29), 151 (12), 79 (9), 136 (8), 78 (7), 51 (7), 53 (6)         |
| 101               | 41.76           | 4-Ethylphenol (t)                                   | H                   | Both      | 120                         | 91 (42), 119 (26), 65 (13), 121 (9), 39 (7), 94 (7), 63 (7), 51 (5), 92 (5)            |
| 102               | 42.04           | 4-(2-Propenyl)phenol                                | H                   | CW        | 134                         | 133 (81), 107 (50), 77 (28), 105 (24), 71 (17), 69 (17), 135 (15), 97 (15), 91 (15)    |
| 104               | 42.59           | Eugenol   | G                   | Both      | 164                         | 149 (33), 103 (26), 131 (25), 77 (24), 91 (21), 137 (19), 133 (17), 104 (16), 121 (14) |
| 105               | 42.71           | 4-Propylguaiacol                                    | G                   | Both      | 137                         | 166 (25), 122 (9), 138 (8), 77 (5), 94 (5), 91 (4), 51 (3), 164 (3), 66 (3)            |
| 113               | 44.97           | 2-Methoxy-4-(1-propenyl)phenol [isoeugenol (cis)]   | G                   | Both      | 164                         | 149 (33), 77 (24), 103 (23), 91 (21), 131 (21), 44 (17), 133 (14), 55 (14), 121 (12)   |
| 116               | 46.50           | 4-Propenylphenol (trans)                            | H                   | Both      | 134                         | 133 (79), 107 (28), 105 (25), 77 (19), 115 (13), 91 (12), 79 (10), 135 (10), 119 (9)   |
| 119               | 47.23           | 2-Methoxy-4-(1-propenyl)phenol [isoeugenol (trans)] | G                   | Both      | 164                         | 149 (32), 77 (21), 103 (21), 131 (20), 91 (20), 133 (13), 55 (12), 121 (12), 165 (11)  |
| 122               | 48.07           | Vanillin  | G                   | Both      | 151                         | 152 (91), 81 (20), 109 (15), 123 (15), 53 (8), 153 (8), 51 (8), 52 (8), 108 (6)        |
| 126               | 50.41           | Homovanillin  | G                   | Both      | 137                         | 166 (26), 122 (19), 94 (10), 138 (9), 51 (5), 77 (5), 116 (5), 66 (5), 65 (4)          |
| 127               | 50.76           | 4-Propylphenol                                      | H                   | CW        | 107                         | 136 (22), 77 (21), 108 (9), 78 (7), 51 (7), 164 (6), 45 (6), 79 (6), 73 (5)            |
| 130               | 51.24           | Acetoguaiacone                                      | G                   | Both      | 151                         | 166 (50), 123 (21), 152 (9), 108 (9), 43 (7), 52 (6), 65 (6), 77 (5), 167 (5)          |
| 131               | 51.57           | (1,6)-Anhydrogalactopyranose                        | Ps                  | CW        | 60                          | 73 (67), 57 (49), 29 (22), 56 (18), 43 (18), 42 (16), 55 (15), 74 (14), 71 (14)        |
| 134               | 52.46           | 4-Hydroxybenzaldehyde                               | Ph                  | CW        | 121                         | 122 (84), 93 (34), 65 (28), 39 (14), 63 (9), 123 (7), 66 (6), 62 (5), 38 (5)           |
| 135               | 53.18           | Guaiacylacetone                                     | G                   | Both      | 137                         | 180 (22), 122 (15), 138 (10), 94 (7), 43 (7), 77 (3), 51 (3), 66 (3), 65 (3)           |
| 138               | 54.46           | Propioguaiacone                                     | G                   | Both      | 151                         | 180 (26), 107 (15), 123 (14), 152 (9), 108 (8), 77 (8), 51 (5), 52 (5), 135 (5)        |
| 145               | 56.67           | (1,4)-Anhydrogalactopyranose (t)                    | Ps                  | Both      | 73                          | 87 (58), 71 (55), 69 (54), 70 (44), 57 (44), 60 (43), 29 (39), 43 (38), 31 (35)        |
| 146               | 57.27           | (1,6)-Anhydroglucopyranose (levoglucosan)           | Ps                  | Both      | 60                          | 57 (43), 73 (38), 29 (18), 70 (16), 56 (16), 43 (15), 42 (14), 55 (13), 31 (11)        |
| 147               | 57.92           | Dihydroconiferyl alcohol                            | G                   | Both      | 137                         | 182 (41), 138 (32), 122 (13), 77 (9), 123 (8), 91 (8), 106 (7), 79 (5), 107 (5)        |
| 148               | 58.93           | Coniferyl alcohol (cis)                             | G                   | Both      | 137                         | 180 (61), 124 (47), 91 (32), 119 (22), 77 (16), 103 (15), 147 (13), 109 (13), 65 (11)  |
| 150               | 60.71           | Unknown anhydrosugar                                | Ps                  | Both      | 73                          | 45 (92), 69 (85), 41 (71), 91 (62), 115 (30), 42 (23), 114 (21), 164 (18), 27 (17)     |
| 151               | 61.89           | Coniferyl alcohol (trans)                           | G                   | Both      | 137                         | 180 (72), 124 (50), 91 (34), 119 (23), 77 (17), 103 (15), 147 (15), 131 (14), 65 (12)  |
| 152               | 62.54           | Coniferaldehyde                                     | G                   | Both      | 178                         | 135 (39), 147 (36), 107 (28), 77 (28), 177 (25), 161 (19), 89 (15), 118 (14), 109 (14) |
| 153               | 63.89           | Unknown   | L                   | CW        | 147                         | 148 (95), 91 (48), 119 (38), 120 (31), 131 (24), 89 (15), 39 (14), 51 (10), 92 (10)    |

<sup>a</sup>Peak refers to the peak number as displayed in the chromatogram figures.<sup>b</sup>RT, retention time (minutes).<sup>c</sup>(t), Tentative assignment.<sup>d</sup>G, G-unit pyrolysis product; H, H-unit pyrolysis product; Ph, pyrolysis products of either H-units or polysaccharides that are phenolic compounds with no methoxyl group and no, or a single carbon, sidechain; Ps, polysaccharide pyrolysis product; L, unknown lignin pyrolysis product.<sup>e</sup>Base ion is the most abundant ion (relative intensity=100%), the m/z of the next nine most abundant ions are shown, with their relative intensities in parentheses.



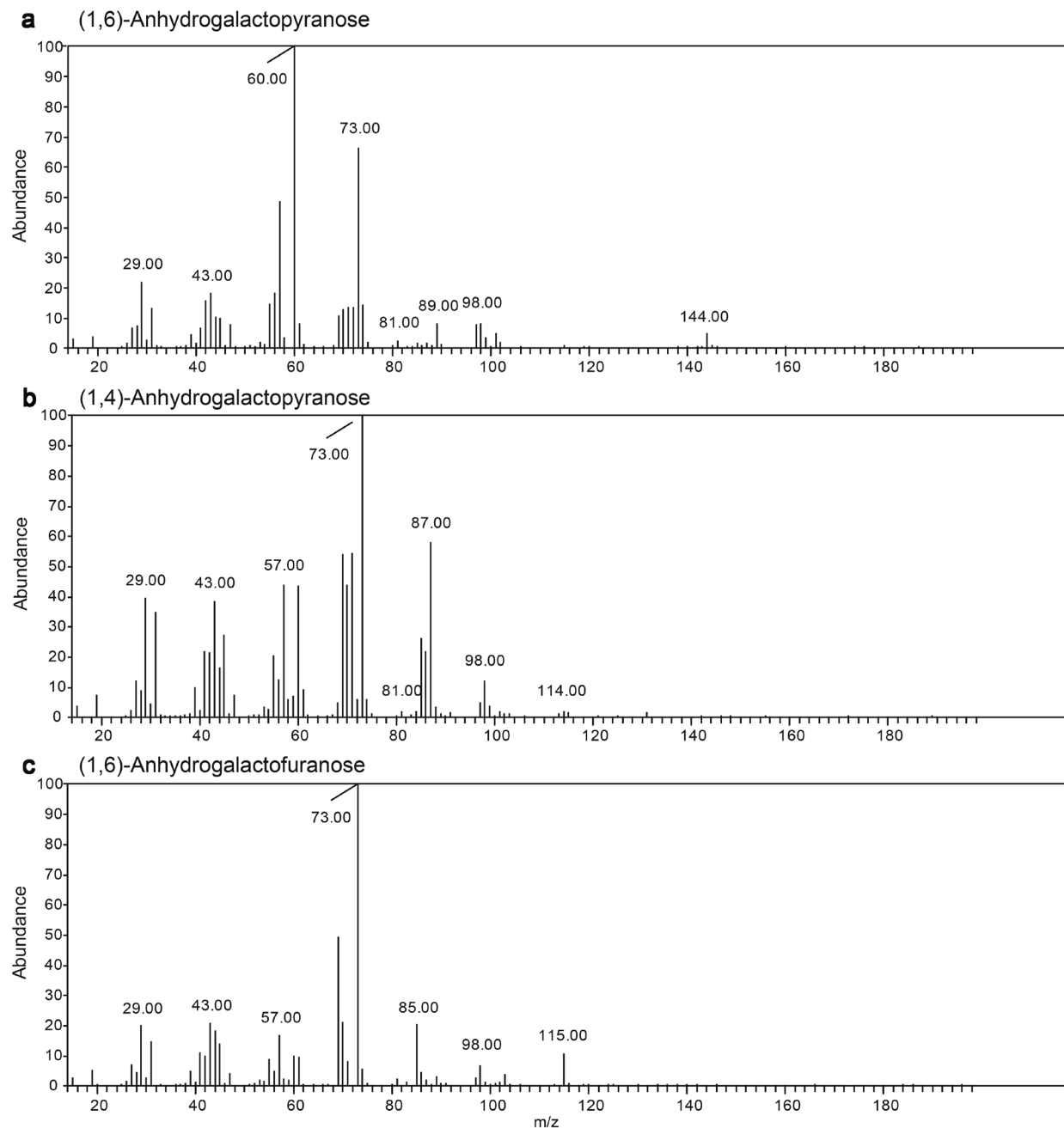
**Figure 1** Baseline-corrected pyrolysis chromatograms of  $\text{CaCl}_2$ -washed (a) CW and (b) OW. Peak numbers correspond to the products listed in Table 1.



**Figure 2** (a) 4-propenylguaiacol and (b) 4-propenylphenol derived from G- and H-units of lignin, respectively, for demonstration of the traditional nomenclature of lignin chemistry.

## Statistical analyses of the pyrograms

Data were analysed in the open-source software R (R Development Core Team 2011). One way analysis of variance (ANOVA) tests were used to test the significance ( $\alpha=0.05$ ) of differences in proportions of each of the pyrolysis products between pre-treatments within wood types and the significance of differences in proportions of each of the pyrolysis products between samples within pre-treatments within wood types. When a significant difference was found between the categorical predictor variable, post hoc Tukey's HSD tests were used to determine which levels of that variable were significantly ( $\alpha=0.05$ )



**Figure 3** Mass spectra of the pyrolysis products from a preparation of lupin seed (1 $\rightarrow$ 4)- $\beta$ -D-galactan. (a) (1,6)-Anhydrogalactopyranose, (b) (1,4)-anhydrogalactopyranose, and (c) (1,6)-anhydrogalactofuranose.



**Table 2** Differences in proportions of pyrolysis products among different pre-treatments of CW samples.

| Peak <sup>a</sup> | Identification <sup>b</sup>                         | Origin <sup>c</sup> | Coarse <sup>d</sup> | Fine <sup>d</sup> | Calcium <sup>d</sup> |
|-------------------|---|---------------------|---------------------|-------------------|----------------------|
| 65                | Phenol  | Ph                  | 0.59 a              | 0.87 ab           | 0.32 b               |
| 66                | Guaiacol [2-methoxyphenol]                          | G                   | 2.99 a              | 2.73 a            | 1.27 b               |
| 69                | 2-Methylphenol                                      | Ph                  | 0.17                | 0.16              | 0.16                 |
| 77                | 4-Methylphenol                                      | Ph                  | 0.37                | 0.34              | 0.45                 |
| 78                | 3-Methylphenol                                      | Ph                  | 0.13                | 0.13              | 0.14                 |
| 83                | 4-Methylguaiacol                                    | G                   | 1.62 a              | 1.43 a            | 2.14 b               |
| 86                | 3,4-Dimethylphenol                                  | Ph                  | 0.13 a              | 0.12 a            | 0.21 b               |
| 88                | 4-Ethylphenol                                       | H                   | 0.07                | 0.03              | 0.07                 |
| 89                | 3-Ethylguaiacol                                     | G                   | 0.06                | 0.04              | 0.09                 |
| 91                | 4-Ethylguaiacol                                     | G                   | 0.37                | 0.31              | 0.33                 |
| 99                | (1,5)-Anhydroarabinofuranose                        | Ps                  | 0.29 a              | 0.25 a            | 0.44 b               |
| 100               | 4-Ethenylguaiacol                                   | G                   | 3.47 a              | 3.18 b            | 2.18 c               |
| 101               | 4-Ethenylphenol (t)                                 | H                   | 0.47                | 0.47              | 0.35                 |
| 102               | 4-(2-Propenyl)phenol                                | H                   | 0.10                | 0.09              | 0.11                 |
| 104               | Eugenol   | G                   | 0.58                | 0.52              | 0.57                 |
| 105               | 4-Propylguaiacol                                    | G                   | 0.13 a              | 0.11 ab           | 0.11 b               |
| 113               | 2-Methoxy-4-(1-propenyl)phenol [isoeugenol (cis)]   | G                   | 0.62 a              | 0.57 ab           | 0.51 b               |
| 116               | 4-Propenylphenol (trans)                            | H                   | 0.31                | 0.28              | 0.37                 |
| 119               | 2-Methoxy-4-(1-propenyl)phenol [isoeugenol (trans)] | G                   | 2.28                | 2.10              | 2.07                 |
| 122               | Vanillin  | G                   | 1.23 a              | 1.14 a            | 1.54 b               |
| 126               | Homovanillin  | G                   | 0.59 a              | 0.55 a            | 0.93 b               |
| 127               | 4-Propylphenol                                      | H                   | 0.23 a              | 0.18 ab           | 0.14 b               |
| 130               | Acetoguaiacone                                      | G                   | 0.72 a              | 0.65 a            | 0.92 b               |
| 131               | (1,6)-Anhydrogalactopyranose                        | Ps                  | 0.00 a              | 0.01 a            | 0.16 b               |
| 134               | 4-Hydroxybenzaldehyde                               | Ph                  | 0.13                | 0.11              | 0.12                 |
| 135               | Guaiacylacetone                                     | G                   | 0.50 a              | 0.50 a            | 0.26 b               |
| 138               | Propioguaiacone                                     | G                   | 0.11 a              | 0.10 a            | 0.19 b               |
| 145               | (1,4)-Anhydrogalactopyranose (t)                    | Ps                  | 0.04 a              | 0.05 a            | 0.59 b               |
| 146               | (1,6)-Anhydroglucopyranose (levoglucosan)           | Ps                  | 1.51 a              | 1.26 a            | 8.01 b               |
| 147               | Dihydroconiferyl alcohol                            | G                   | 0.27                | 0.23              | 0.22                 |
| 148               | Coniferyl alcohol (cis)                             | G                   | 0.38 a              | 0.33 a            | 0.20 b               |
| 150               | Unknown anhydrosugar                                | Ps                  | 0.11 a              | 0.10 b            | 1.86 c               |
| 151               | Coniferyl alcohol (trans)                           | G                   | 2.64 a              | 1.93 a            | 0.64 b               |
| 152               | Coniferaldehyde                                     | G                   | 1.95                | 2.00              | 1.97                 |
| 153               | Unknown   | L                   | 0.14                | 0.11              | 0.12                 |

<sup>a</sup>Peak refers to the peak number as displayed in the chromatogram figures.

<sup>b</sup>(t), Tentative assignment.

<sup>c</sup>G, G-unit pyrolysis product; H, H-unit pyrolysis product; Ph, pyrolysis products of either H-units or polysaccharides that are phenolic compounds with no methoxyl group and no, or a single carbon, sidechain; Ps, polysaccharide pyrolysis product; L, unknown lignin pyrolysis product.

<sup>d</sup>Values are the mean proportion of the total integratable area (%) of six samples of each wood type. Significant differences between treatments are denoted by lower case letters after the proportion of the product (tested by post-hoc Tukey's HSD tests).

different from each other. For products common to both wood types after CaCl<sub>2</sub> treatment, a Welch's independent sample t-test was used to determine if there was a significant ( $\alpha=0.05$ ) difference in the proportion of each of the products between wood types.

## Results and discussion

### Pyrolysis products from CW and OW

The retention times, mass spectra, and identification of pyrolysis products in the time range of interest are listed

in Table 1. Typical pyrograms of CW and OW after baseline correction are presented in Figure 1 and the peak numbers correspond to those in Table 1. The basic structure of typical softwood lignin-derived pyrolysis products are depicted in Figure 2.

Phenolic pyrolysis products with a C<sub>6</sub>- [65] or a C<sub>6</sub>-C<sub>1</sub> skeleton without OMe are mainly from H-lignin (Bremer 1991), but the formation of phenols in small amounts from polysaccharides cannot be excluded (Pouwels et al. 1989; Ralph and Hatfield 1991; Rencoret et al. 2011). To reflect this possibility, such products (e.g., phenol [65], methylphenols [69, 77, 78] and 4-hydroxybenzaldehyde [134])

**Table 3** Differences in proportions of pyrolysis products among different pre-treatments of OW samples.

| Peak <sup>a</sup> | Identification <sup>b</sup>                         | Origin <sup>c</sup> | Coarse <sup>d</sup> | Fine <sup>d</sup> | Calcium <sup>d</sup> |
|-------------------|---|---------------------|---------------------|-------------------|----------------------|
| 65                | Phenol  | Ph                  | 0.17 a              | 0.23 b            | 0.10 c               |
| 66                | Guaiacol [2-methoxyphenol]                          | G                   | 2.41 a              | 2.24 a            | 0.99 b               |
| 69                | 2-Methylphenol                                      | Ph                  | 0.17 a              | 0.10 b            | 0.12 b               |
| 77                | 4-Methylphenol                                      | Ph                  | 0.12 a              | 0.11 ab           | 0.09 b               |
| 78                | 3-Methylphenol                                      | Ph                  | 0.08 a              | 0.08 ab           | 0.06 b               |
| 83                | 4-Methylguaiacol                                    | G                   | 1.27 a              | 1.09 b            | 1.65 c               |
| 86                | 3,4-Dimethylphenol                                  | Ph                  | 0.09 a              | 0.09 a            | 0.15 b               |
| 89                | 3-Ethylguaiacol                                     | G                   | 0.02 a              | 0.01 a            | 0.07 b               |
| 91                | 4-Ethylguaiacol                                     | G                   | 0.34 a              | 0.25 b            | 0.26 b               |
| 99                | (1,5)-Anhydroarabinofuranose                        | Ps                  | 0.47 a              | 0.39 a            | 0.68 b               |
| 100               | 4-Ethenylguaiacol                                   | G                   | 3.58 a              | 3.14 b            | 1.88 c               |
| 101               | 4-Ethenylphenol (t)                                 | H                   | 0.09 a              | 0.09 a            | 0.06 b               |
| 104               | Eugenol   | G                   | 0.54 a              | 0.46 b            | 0.50 b               |
| 105               | 4-Propylguaiacol                                    | G                   | 0.12 a              | 0.10 b            | 0.10 b               |
| 113               | 2-Methoxy-4-(1-propenyl)phenol [isoeugenol (cis)]   | G                   | 0.54 a              | 0.48 b            | 0.46 b               |
| 116               | 4-Propenylphenol (trans)                            | H                   | 0.01 a              | 0.00 a            | 0.06 b               |
| 119               | 2-Methoxy-4-(1-propenyl)phenol [isoeugenol (trans)] | G                   | 2.18 a              | 1.81 a            | 1.93 b               |
| 122               | Vanillin  | G                   | 0.99 a              | 0.91 a            | 1.22 b               |
| 126               | Homovanillin  | G                   | 0.40 a              | 0.36 a            | 0.55 b               |
| 130               | Acetoguaiacone                                      | G                   | 0.60 a              | 0.51 b            | 0.69 c               |
| 135               | Guaiacylacetone                                     | G                   | 0.47 a              | 0.44 a            | 0.22 b               |
| 138               | Propioguaiacone                                     | G                   | 0.07 a              | 0.06 a            | 0.19 b               |
| 145               | (1,4)-Anhydrogalactopyranose (t)                    | Ps                  | 0.14                | 0.00              | 0.05                 |
| 146               | (1,6)-Anhydroglucopyranose (levoglucosan)           | Ps                  | 1.41 a              | 1.39 a            | 9.56 b               |
| 147               | Dihydroconiferyl alcohol                            | G                   | 0.40 a              | 0.36 ab           | 0.32 b               |
| 148               | Coniferyl alcohol (cis)                             | G                   | 0.27 a              | 0.27 a            | 0.13 b               |
| 150               | Unknown anhydrosugar                                | Ps                  | 0.24 a              | 0.12 a            | 1.93 b               |
| 151               | Coniferyl alcohol (trans)                           | G                   | 1.91 a              | 1.52 b            | 0.33 c               |
| 152               | Coniferaldehyde                                     | G                   | 1.63                | 1.68              | 1.58                 |

<sup>a</sup>Peak refers to the peak number as displayed in the chromatogram figures.

<sup>b</sup>(t), Tentative assignment.

<sup>c</sup>G, G-unit pyrolysis product; H, H-unit pyrolysis product; Ph, pyrolysis products of either H-units or polysaccharides that are phenolic compounds with no methoxyl group and no, or a single carbon, sidechain; Ps, polysaccharide pyrolysis product; L, unknown lignin pyrolysis product.

<sup>d</sup>Values are the mean proportion of the total integratable area (%) of six samples of each wood type. Significant differences between treatments are denoted by lower case letters after the proportion of the product (tested by post-hoc Tukey's HSD tests).

have been identified as being derived from H-lignin or polysaccharides (Table 1). Products of the type C<sub>6</sub>-C<sub>2</sub> or C<sub>6</sub>-C<sub>3</sub> (e.g., 4-ethylphenol [88], 4-ethenylphenol [101], 4-(2-propenyl)-phenol [102], 4-propenylphenol [116] and 4-propylphenol [127], Table 1) are typical for lignin.

Typical pyrolysis products from polysaccharides result from cleavage of the glycosidic bonds followed by the formation of anhydrosugars from the monosaccharides. Pyrolysis of OW and CW give rise to: (1,5)-anhydroarabinofuranose [99], (1,4)-anhydrogalactopyranose [145] and (1,6)-anhydroglucopyranose (levoglucosan) [146]. (1,6)-Anhydrogalactopyranose [131] was only produced in detectable amounts in the pyrograms of CW. Pyrolysis of lupin seed (1→4)-β-D-galactan produced (1,6)-anhydrogalactopyranose, (1,6)-anhydrogalactofuranose, and a product that is probably

(1,4)-anhydrogalactopyranose (see mass spectra in Figure 3). The preliminary identification of (1,4)-anhydrogalactopyranose is based on retention time data of Budgell et al. (1987) obtained on the same GC column and related to (1,6)-anhydrogalactopyranose and (1,6)-anhydrogalactofuranose. MS data are not available from the literature.

## Influence of sample preparation

The effects of the three pre-treatments on pyrolysis are compiled in Table 2 (CW) and Table 3 (OW). The total amount of non-specific compounds (acetic acid and furfurals) typically increased upon fine-milling of both CW and OW (data not shown). Fine-milling of the CW



**Table 4** Differences in proportions, between samples of pyrolysis products that were unique to CW.

| Peak <sup>a</sup> | Identification               | Origin <sup>b</sup> | CW 1 (SD)     | CW 2 (SD)     | CW 3 (SD)     | CW 4 (SD)     | CW 5 (SD)     | CW 6 (SD)     | F    | P-value |
|-------------------|------------------------------|---------------------|---------------|---------------|---------------|---------------|---------------|---------------|------|---------|
| 88                | 4-Ethylphenol                | H                   | 0.056 (0.002) | 0.078 (0.002) | 0.069 (0.002) | 0.040 (0.035) | 0.082 (0.009) | 0.074 (0.003) | 3.3  | 0.0418  |
| 102               | 4-(2-Propenyl)phenol         | H                   | 0.097 (0.021) | 0.125 (0.010) | 0.109 (0.014) | 0.095 (0.005) | 0.133 (0.007) | 0.130 (0.010) | 5.6  | 0.007   |
| 127               | 4-Propylphenol               | H                   | 0.118 (0.007) | 0.182 (0.016) | 0.115 (0.018) | 0.109 (0.008) | 0.164 (0.026) | 0.143 (0.013) | 10.2 | 0.0005  |
| 131               | (1,6)-Anhydrogalactopyranose | Ps                  | 0.035 (0.061) | 0.206 (0.023) | 0.138 (0.021) | 0.045 (0.041) | 0.269 (0.125) | 0.270 (0.085) | 6.9  | 0.003   |
| 134               | 4-Hydroxybenzaldehyde        | Ph                  | 0.089 (0.004) | 0.144 (0.005) | 0.114 (0.006) | 0.075 (0.006) | 0.173 (0.013) | 0.133 (0.011) | 59.1 | <0.0001 |
| 153               | Unknown                      | L                   | 0.072 (0.014) | 0.143 (0.020) | 0.119 (0.004) | 0.085 (0.003) | 0.169 (0.021) | 0.132 (0.010) | 20.1 | <0.0001 |

Values are the mean proportion of the total integratable area (%) of three replicates of six samples of CW, standard deviation is given in parentheses. The F value and P-value are given for one-way ANOVA for the effect of sample on the proportion of each product. Degrees of freedom are the following: sample=5, residuals=12.

<sup>a</sup>Peak refers to the peak number as displayed in the chromatogram figures.

<sup>b</sup>H, H-unit pyrolysis product; Ph, pyrolysis products of either H-units or polysaccharides that are phenolic compounds with no methoxyl group and no, or a single carbon, sidechain; Ps, polysaccharide pyrolysis product; L, unknown lignin pyrolysis product.

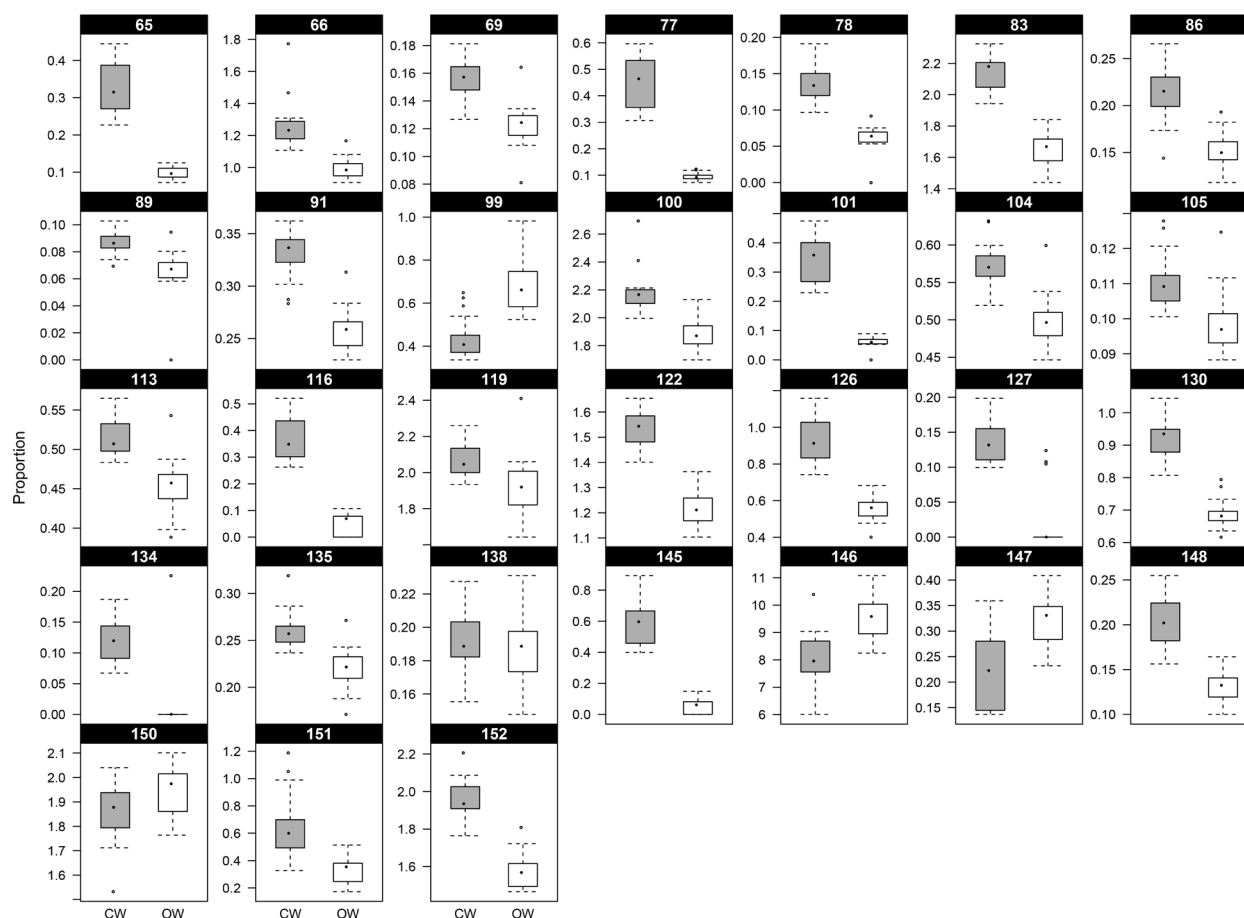
samples significantly reduced only the proportions of 4-ethenylguaiaicol [100], and the unknown anhydrosugar at retention time 60.71 min (Table 1, [150]). Conversely, fine-milling of the OW samples had a significant effect, for example, the yield of several phenolic pyrolysis products decreased and the proportion of phenol increased. The yield of anhydrosugars was not affected by fine-milling.

Ikedo et al. (2001) have shown that dry-milling (under liquid N<sub>2</sub>) of loblolly pine (*Pinus taeda*) wood causes depolymerisation and promotes the formation of low molecular weight products. Syverud et al. (2003) found that milling at room temperature of both softwood and hardwood pulps leads to lower yield of anhydrosugars in the pyrograms. It may be assumed that the fine-milled samples, with their high surface area, pyrolysed more rapidly and completely to lower molecular weight products, which are non-specific. However, absolute quantification of pyrolysis products is difficult in the absence of an internal standard.

For both CW and OW, CaCl<sub>2</sub>-treatment did not have a uniform effect on the lignin-derived products. The yield of 4-methylguaiaicol [83] and vanillin [122] increased, for example, whereas those of phenol [65] and 4-ethenylguaiaicol [100] decreased. Kleen and Gellerstedt (1995) found greater proportions of anhydrosugars with CaCl<sub>2</sub>-washed samples. Such an increase was also observed in our data for both CW and OW. CaCl<sub>2</sub>-treatment of OW samples increased the yields of (1,5)-anhydroarabinofuranose [99], (1,6)-anhydroglucopyranose (levoglucosan) [146], and the unknown anhydrosugar [150]. CaCl<sub>2</sub>-treatment of CW samples increased the yields of these three sugars, and also the galactose-derived (1,6)-anhydrogalactopyranose [131] and (1,4)-anhydrogalactopyranose [145]. This finding is also consistent with those of Syverud et al. (2003), who found improved yields of galactose-, xylose-, mannose- and arabinose-derived pyrolysis products from pulps after exchanging sodium with calcium. In summary, fine-milled and CaCl<sub>2</sub>-treated samples are the best candidates for distinguishing CW from OW as the typical products from (1→4)-β-D-galactans are more pronounced. In the following sections, only the results of CaCl<sub>2</sub>-treated samples will be discussed.

## Typical markers of CW

Six pyrolysis products were unique to CW, most of which were due to H-units in CW lignin. The following products did not occur in any OW samples: 4-(2-propenyl)



**Figure 4** Boxplots showing the proportions of the pyrolysis products (numbers refer to peak numbers) from samples of  $\text{CaCl}_2$ -treated CW and samples of  $\text{CaCl}_2$ -treated OW. Welch's t-test indicated a significant difference of degradation product yields obtained from CW and OW except for propioguuaiacone [138]. For each wood type, there were three replicates of six samples.

phenol [102], (1,6)-anhydrogalactopyranose [131], and an unknown lignin-derived product (Table 1, [153]). Other products were present in small proportions in only one portion of one OW sample (4-ethylphenol [88]), or in small proportions in only one portion of two OW samples (4-propylphenol [127] and 4-hydroxybenzaldehyde [134]). Their occurrence in OW could be experimental artefacts due to contamination, or these products were below the limits of detection in the other OW samples. The relative proportions of the six CW markers in the current study were significantly different among some of the compression wood samples (Table 4), indicating that they might be useful in distinguishing among samples with differing degrees of compression wood severity.

Mast et al. (2009) and Nanayakkara (2007) examined CW and OW of radiata pine by Py-GC/MS. The results of the quoted authors also indicated that the relative proportions of the six unique CW markers identified in our study were greater in CW compared to OW, but that they were not unique to CW. However, the peaks in the chromatograms

were not well resolved from each other, and other pyrolysis products may have contributed to those peaks. Alves et al. (2009) successfully distinguished CW from NW samples by principal component analysis of pyrolysis products derived from H-units and G-units of lignin.

## Quantification of Py products in CW and OW

The range of proportions of the pyrolysis products from CW and OW samples are shown in a boxplot in Figure 4. The yields of pyrolysis products common to both wood types were nearly all significantly different between CW and OW (Table 5). The G-unit-derived product propioguuaiacone [138] was the only exception ( $P=0.43$ ). The proportions of lignin-derived compounds were typically greater in CW than OW, consistent with the concept that CW of radiata pine contains more lignin (Nanayakkara et al. 2009; Brennan et al. 2012). Dihydroconiferyl alcohol [147] was an exception with its higher intensity in pyrograms of OW compared

**Table 5** Differences in the proportions of pyrolysis products between CW and OW that are common to both wood types.

| Peak <sup>a</sup> | Identification <sup>b</sup>                          | Origin <sup>c</sup> | CW (SD)     | OW (SD)     | $\Delta\bar{X}$ <sup>d</sup> | P-value <sup>e</sup> |
|-------------------|--|---------------------|-------------|-------------|------------------------------|----------------------|
| 65                | Phenol   | Ph                  | 0.32 (0.07) | 0.10 (0.01) | 0.23                         | <0.0001              |
| 66                | Guaiacol [2-methoxyphenol]                           | G                   | 1.27 (0.12) | 0.99 (0.04) | 0.27                         | <0.0001              |
| 69                | 2-Methylphenol                                       | Ph                  | 0.16 (0.01) | 0.12 (0.01) | 0.03                         | <0.0001              |
| 77                | 4-Methylphenol                                       | Ph                  | 0.45 (0.10) | 0.09 (0.01) | 0.36                         | <0.0001              |
| 78                | 3-Methylphenol                                       | Ph                  | 0.14 (0.02) | 0.06 (0.01) | 0.07                         | <0.0001              |
| 83                | 4-Methylguaiacol                                     | G                   | 2.14 (0.11) | 1.65 (0.09) | 0.49                         | <0.0001              |
| 86                | 3,4-Dimethylphenol                                   | Ph                  | 0.21 (0.02) | 0.15 (0.01) | 0.06                         | <0.0001              |
| 89                | 3-Ethylguaiacol                                      | G                   | 0.09 (0.01) | 0.07 (0.01) | 0.02                         | 0.0009               |
| 91                | 4-Ethylguaiacol                                      | G                   | 0.33 (0.02) | 0.26 (0.02) | 0.07                         | <0.0001              |
| 99                | (1,5)-Anhydroarabinofuranose                         | Ps                  | 0.44 (0.10) | 0.68 (0.10) | 0.24                         | <0.0001              |
| 100               | 4-Ethenylguaiacol                                    | G                   | 2.18 (0.12) | 1.88 (0.07) | 0.31                         | <0.0001              |
| 101               | 4-Ethenylphenol (t)                                  | H                   | 0.35 (0.08) | 0.06 (0.02) | 0.30                         | <0.0001              |
| 104               | Eugenol  | G                   | 0.57 (0.02) | 0.5 (0.02)  | 0.07                         | <0.0001              |
| 105               | 4-Propylguaiacol                                     | G                   | 0.11 (0.01) | 0.1 (0.01)  | 0.01                         | 0.0007               |
| 113               | Isoeugenol (cis) [2-Methoxy-4-(1-propenyl)-phenol]   | G                   | 0.51 (0.01) | 0.46 (0.03) | 0.06                         | <0.0001              |
| 116               | 4-Propenylphenol (trans)                             | H                   | 0.37 (0.08) | 0.06 (0.03) | 0.31                         | <0.0001              |
| 119               | Isoeugenol (trans) [2-Methoxy-4-(1-propenyl)-phenol] | G                   | 2.07 (0.05) | 1.93 (0.13) | 0.14                         | 0.0049               |
| 122               | Vanillin   | G                   | 1.54 (0.07) | 1.22 (0.05) | 0.32                         | <0.0001              |
| 126               | Homovanillin   | G                   | 0.93 (0.13) | 0.55 (0.05) | 0.38                         | <0.0001              |
| 130               | Acetoguaiacone                                       | G                   | 0.92 (0.05) | 0.69 (0.03) | 0.23                         | <0.0001              |
| 135               | Guaiacylacetone                                      | G                   | 0.26 (0.02) | 0.22 (0.01) | 0.04                         | <0.0001              |
| 138               | Propioguaiacone                                      | G                   | 0.19 (0.01) | 0.19 (0.01) | 0.01                         | 0.4277               |
| 145               | (1,4)-Anhydrogalactopyranose (t)                     | Ps                  | 0.59 (0.13) | 0.05 (0.03) | 0.54                         | <0.0001              |
| 146               | (1,6)-Anhydroglucopyranose (levoglucosan)            | Ps                  | 8.01 (0.96) | 9.56 (0.64) | 1.55                         | <0.0001              |
| 147               | Dihydroconiferyl alcohol                             | G                   | 0.22 (0.07) | 0.32 (0.05) | 0.09                         | <0.0001              |
| 148               | Coniferyl alcohol (cis)                              | G                   | 0.20 (0.02) | 0.13 (0.01) | 0.07                         | <0.0001              |
| 150               | Unknown anhydrosugar                                 | Ps                  | 1.86 (0.08) | 1.93 (0.06) | 0.08                         | 0.0308               |
| 151               | Coniferyl alcohol (trans)                            | G                   | 0.64 (0.13) | 0.33 (0.04) | 0.31                         | <0.0001              |
| 152               | Coniferaldehyde                                      | G                   | 1.97 (0.09) | 1.58 (0.08) | 0.38                         | <0.0001              |

Values are the mean proportion of the total integratable area (%) of six samples of each wood type, standard deviation (SD) is given in parentheses.

<sup>a</sup>Peak refers to the peak number as displayed in the chromatogram figures.

<sup>b</sup>(t), Tentative assignment.

<sup>c</sup>G, G-unit pyrolysis product; H, H-unit pyrolysis product; Ph, pyrolysis products of either H-units or polysaccharides that are phenolic compounds with no methoxyl group and no, or a single carbon, sidechain; Ps, polysaccharide pyrolysis product.

<sup>d</sup> $\Delta\bar{X}$ , Sample mean difference between CW and OW.

<sup>e</sup>The P-value for Welch's independent sample t-test to determine significant differences between wood types ( $\alpha=0.05$ ) is given.

with that of CW (mean difference=0.09,  $P<0.0001$ ), which was unusual for a lignin-derived product.

The proportions of (1,5)-anhydroarabinofuranose [99] ( $P<0.0001$ ), (1,4)-anhydrogalactopyranose [145] ( $P<0.0001$ ) and (1,6)-anhydroglucopyranose (levoglucosan) [146] ( $P<0.0001$ ) were different between CW and OW. As the pyrolysis of arabinose is efficient relative to the other cell-wall monosaccharides (Ponder et al. 1990; Ralph and Hatfield 1991), it is not surprising that differences in the yields of (1,5)-anhydroarabinofuranose [99] between CW and OW were measureable, despite the fact that arabinosyl residues occur in small amounts in similar wood samples (Brennan et al. 2012). The proportion of (1,4)-anhydrogalactopyranose [145] was greater in CW than that of (1,6)-anhydrogalactopyranose [131] (Table

2), although a previous study on pyrolysis of radiata pine CW reported only the production of (1,6)-anhydrogalactopyranose (Mast et al. 2009). Pyrolysis of apple and citrus pectins, having high proportions of (1→4)- $\beta$ -D-galactan sidechains in rhamnogalacturonan I (RG I) (Renard et al. 1995), yield (1,4)-anhydrogalactopyranose, but not (1,6)-anhydrogalactopyranose and (1,6)-anhydrogalactofuranose (Moldoveanu 1998).

Galactose is also a component of other polysaccharides in the cell walls of wood (Harris 2005). However, it is likely that the increased proportions of (1,4)-anhydrogalactopyranose [145] and (1,6)-anhydrogalactopyranose [131] in CW compared with OW are due to increased proportions of (1→4)- $\beta$ -D-galactans, as radiata pine CW contains a larger proportion of galactosyl residues in (1→4)- $\beta$ -D-galactans

**Table 6** Differences among samples of CW and among samples of OW.

| Peak <sup>a</sup> | Identification <sup>b</sup>                          | Origin <sup>c</sup> | CW      |         |             | OW      |         |             |
|-------------------|--|---------------------|---------|---------|-------------|---------|---------|-------------|
|                   |  |                     | F-value | P-value | Differences | F-value | P-value | Differences |
| 65                | Phenol   | Ph                  | 10.9    | 0.0003  | 7           | 1.26    | 0.3399  | NA          |
| 66                | Guaiacol [2-methoxyphenol]                           | G                   | 3.33    | 0.0403  | 1           | 1.40    | 0.2903  | NA          |
| 69                | 2-Methylphenol                                       | Ph                  | 0.73    | 0.6090  | NA          | 2.57    | 0.0836  | NA          |
| 77                | 4-Methylphenol                                       | Ph                  | 52.6    | <0.0001 | 12          | 4.76    | 0.0125  | 2           |
| 78                | 3-Methylphenol                                       | Ph                  | 4.76    | 0.0124  | 2           | 1.29    | 0.3286  | NA          |
| 83                | 4-Methylguaiacol                                     | G                   | 10.0    | 0.0005  | 5           | 4.21    | 0.0192  | 1           |
| 86                | 3,4-Dimethylphenol                                   | Ph                  | 2.45    | 0.0943  | NA          | 0.65    | 0.6657  | NA          |
| 89                | 3-Ethylguaiacol                                      | G                   | 2.23    | 0.1180  | NA          | 1.56    | 0.2416  | NA          |
| 91                | 4-Ethylguaiacol                                      | G                   | 2.37    | 0.1019  | NA          | 2.67    | 0.0758  | NA          |
| 99                | (1,5)-Anhydroarabinofuranose                         | Ps                  | 17.9    | <0.0001 | 5           | 4.49    | 0.0153  | 3           |
| 100               | 4-Ethenylguaiacol                                    | G                   | 3.17    | 0.0471  | 0           | 1.61    | 0.2288  | NA          |
| 101               | 4-Ethenylphenol (t)                                  | H                   | 16.4    | <0.0001 | 8           | 1.96    | 0.1571  | NA          |
| 104               | Eugenol  | G                   | 0.95    | 0.4826  | NA          | 2.00    | 0.1501  | NA          |
| 105               | 4-Propylguaiacol                                     | G                   | 2.20    | 0.1215  | NA          | 5.48    | 0.0074  | 3           |
| 113               | Isoeugenol (cis) [2-Methoxy-4-(1-propenyl)-phenol]   | G                   | 1.27    | 0.3364  | NA          | 2.52    | 0.0872  | NA          |
| 116               | 4-Propenylphenol (trans)                             | H                   | 19.1    | <0.0001 | 8           | 1.88    | 0.1705  | NA          |
| 119               | Isoeugenol (trans) [2-Methoxy-4-(1-propenyl)-phenol] | G                   | 0.92    | 0.5005  | NA          | 2.72    | 0.0719  | NA          |
| 122               | Vanillin   | G                   | 17.0    | <0.0001 | 9           | 2.04    | 0.1443  | NA          |
| 126               | Homovanillin   | G                   | 10.0    | 0.0005  | 5           | 3.06    | 0.0518  | NA          |
| 130               | Acetoguaiacone                                       | G                   | 8.42    | 0.0012  | 4           | 1.76    | 0.1940  | NA          |
| 135               | Guaiacylacetone                                      | G                   | 3.45    | 0.0363  | 1           | 0.48    | 0.7811  | NA          |
| 138               | Propioguiacone                                       | G                   | 1.85    | 0.1763  | NA          | 0.46    | 0.7975  | NA          |
| 145               | (1,4)-Anhydrogalactopyranose (t)                     | Ps                  | 6.52    | 0.0037  | 4           | 1.45    | 0.2744  | NA          |
| 146               | (1,6)-Anhydroglucopyranose (levoglucosan)            | Ps                  | 6.03    | 0.0051  | 3           | 3.90    | 0.0248  | 2           |
| 147               | Dihydroconiferyl alcohol                             | G                   | 44.4    | <0.0001 | 9           | 8.98    | 0.0009  | 5           |
| 148               | Coniferyl alcohol (cis)                              | G                   | 1.00    | 0.4561  | NA          | 0.77    | 0.5835  | NA          |
| 150               | Unknown anhydrosugar                                 | Ps                  | 1.58    | 0.2380  | NA          | 1.08    | 0.4155  | NA          |
| 151               | Coniferyl alcohol (trans)                            | G                   | 0.83    | 0.5472  | NA          | 0.37    | 0.8585  | NA          |
| 152               | Coniferaldehyde                                      | G                   | 5.60    | 0.0068  | 3           | 3.31    | 0.0413  | 1           |

The *F*-value and *P*-value are given for one-way ANOVA for the effect of sample on the proportion of each product within each wood type. Degrees of freedom are the following: sample=5, residuals=12. Differences indicate the number of Tukey's HSD pairwise sample comparisons that were significantly different from each other (out of a possible 15) for that wood type. NA, there were no significant differences among samples in that wood type.

<sup>a</sup>Peak refers to the peak number as displayed in the chromatogram figures.

<sup>b</sup>(t), Tentative assignment.

<sup>c</sup>G, G-unit pyrolysis product; H, H-unit pyrolysis product; Ph, pyrolysis products of either H-units or polysaccharides that are phenolic compounds with no methoxyl group and no, or a single carbon, sidechain; Ps, polysaccharide pyrolysis product.

than in galactoglucomannans, as determined by two-dimensional <sup>13</sup>C-<sup>1</sup>H nuclear magnetic resonance correlation spectroscopy (Brennan et al. 2012), and primary walls make up only a small proportion of the wood mass.

## ANOVA of CW and OW pyrolysis

Results of Tukey's HSD tests to determine differences among samples of each wood type are given in Table 6. Among CW samples, the product 4-methylphenol [77] was present in significantly different proportions in 12 out of a possible 15 pairwise sample comparisons, making this product a likely indicator of CWs with different severities.

Among samples of OW, fewer products were present in significantly different proportions. Dihydroconiferyl alcohol [147] showed the greatest number of differences among samples of OW, at five out of a possible 15 pairwise comparisons. CW samples typically had a greater range in proportions of pyrolysis products than OW samples (Figure 4).

## Detection of lignin H-units in OW

The H-unit type degradation products, 4-ethenylphenol [101] and 4-propenylphenol [116], were detected in low yield in the OW pyrograms. The amount of H-units is small in the "normal" lignin of both straight-grown trees

and OW (Terashima and Fukushima 1988; Yeh et al. 2005, 2006; Tokareva et al. 2007; Nanayakkara et al. 2009). The compound middle lamella (CML, primary wall plus middle lamella) of black spruce (*Picea mariana*) NW was found to have a lower OMe content than the secondary wall fraction (Whiting and Goring 1982), which was interpreted as indicating the presence of H-units. However, Westermarck (1985) suggested that the result could have been due to the presence of small amounts of CW. The presence of a small amount of CW also cannot be excluded in the present paper, although the samples were carefully checked before analysis. The H-units in OW pyrograms could also be from pyrolysis of *p*-coumaric acid esters, which are present in small proportions in primary walls of radiata pine (Sánchez et al. 1996; Carnachan and Harris 2000).

## Conclusions

A number of pyrolysis degradation products from fine-milled and CaCl<sub>2</sub>-treated wood may be useful to detect

compression wood (CW). The majority of them were from the H-units of lignin, which have a higher diagnostic value than those derived from the galactosyl residues in CW. This is probably due to the lower efficiency of carbohydrate pyrolysis, which could not be essentially improved by the CaCl<sub>2</sub>-treatment. Products that were present in different proportions among CW samples should be further investigated as indicators of CW severity.

**Acknowledgments:** We thank our colleagues at the University of Canterbury, Luis Apiolaza, Shakti Chauhan and John Walker, for providing the wood samples, and the New Zealand Foundation for Research Science and Technology (PROJ-12401-PPS-UOC) for funding. We thank Dr. Jürgen Odermatt at the Center of Wood Science and Technology, University of Hamburg, for the use of instruments in his laboratory.

Received June 20, 2013; accepted October 25, 2013; previously published online xx

## References

- Altaner, C.M., Tokareva, E.N., Jarvis, M.C., Harris, P.J. (2010) Distribution of (1→4)-β-galactans, arabinogalactan proteins, xylans and (1→3)-β-glucans in tracheid cell walls of softwoods. *Tree Physiol.* 30:782–793.
- Alves, A., Gierlinger, N., Schwanninger, M., Rodrigues, J. (2009) Analytical pyrolysis as a direct method to determine the lignin content in wood: Part 3. Evaluation of species-specific and tissue-specific differences in softwood lignin composition using principal component analysis. *J. Anal. Appl. Pyrolysis* 85:30–37.
- Apiolaza, L., Chauhan, S., Walker, J. (2011a). Genetic control of very early compression and opposite wood in *Pinus radiata* and its implications for selection. *Tree Genet. Genomes* 7:563–571.
- Apiolaza, L.A., Butterfield, B., Chauhan, S.S., Walker, J.C.F. (2011b). Characterization of mechanically perturbed young stems: can it be used for wood quality screening? *Annals Forest Sci.* 68:407–414.
- Beaumont, O., Schwob, Y. (1984) Influence of physical and chemical parameters on wood pyrolysis. *Ind. Eng. Chem. Process Des. Dev.* 23:637–641.
- Bland, D.E. (1958) The chemistry of reaction wood. Part I. The lignins of *Eucalyptus gonicalyx* and *Pinus radiata*. *Holzforschung* 12:36–43.
- Bouveng, H.O., Meier, H. (1959) Studies on a galactan from Norwegian spruce compression wood (*Picea abies* Karst.). *Acta Chem. Scand.* 13:1884–1889.
- Bremer, J. (1991) Quantifizierung der Gerüstsubstanzen von Lignocellulosen durch analytische Pyrolyse-GC/MS. (In German). Doctoral thesis, Faculty of Biology, University of Hamburg.
- Brennan, M., McLean, J.P., Altaner, C.M., Ralph, J., Harris, P.J. (2012) Cellulose microfibril angles and cell-wall polymers in different wood types of *Pinus radiata*. *Cellulose* 19: 1385–1404.
- Budgell, D.R., Hayes, E.R., Helleur, R.J. (1987) Direct identification of pentoses and hexoses by pyrolysis/capillary gas chromatography. *Anal. Chim. Acta* 192:243–253.
- Butterfield, B.G., Meylan B.A. Three-dimensional Structure of Wood: An Ultrastructural Approach. Chapman and Hall, London, 1980.
- Carnachan, S.M., Harris, P.J. (2000) Polysaccharide compositions of primary cell walls of the palms *Phoenix canariensis* and *Rhopalostylis sapida*. *Plant Physiol. Biochem.* 38:699–708.
- del Río, J.C., Prinsen, P., Rencoret, J., Nieto, L., Jiménez-Barbero, J., Ralph, J., Martínez, Á.T., Gutiérrez, A. (2012) Structural characterization of the lignin in the cortex and pith of elephant grass (*Pennisetum purpureum*) stems. *J. Agric. Food Chem.* 60:3619–3634.
- Donaldson, L.A., Grace, J., Downes, G.M. (2004) Within-tree variation in anatomical properties of compression wood in radiata pine. *IAWA J.* 25:253–271.
- Faix, O., Meier, D., Fortmann, I. (1990) Thermal degradation products of wood. Gas chromatographic separation and mass spectrometric characterization of monomeric lignin derived products. *Holz Roh. Werkst.* 48:281–285.
- Faix, O., Fortmann, I., Bremer, J., Meier, D. (1991) Thermal degradation products of wood. Gas chromatographic separation and mass spectrometric characterization of polysaccharide derived products. *Holz Roh. Werkst.* 49: 213–219.



- Galletti, G.C., Bocchini, P. (1995) Pyrolysis/gas chromatography/mass spectrometry of lignocellulose. *Rapid Commun. Mass Spectrom.* 9:815–826.
- Gur'janov, O.P., Ibragimova, N.N., Gnezdilov, O.I., Gorshkova, T.A. (2008) Polysaccharides, tightly bound to cellulose in cell wall of flax bast fibre: Isolation and identification. *Carbohydr. Polym.* 72:719–729.
- Harris, P.J. (2005) Diversity in plant cell walls. In: *Plant Diversity and Evolution: Genotypic and Phenotypic Variation in Higher Plants*. Ed. R.J. Henry. CAB International Publishing, Wallingford. pp. 201–227.
- Harris, P.J., Stone, B.A. (2008) Chemistry and molecular organization of plant cell walls. In: *Biomass Recalcitrance: Deconstructing the Plant Cell Wall for Bioenergy*. Ed. M.E. Himmel. Blackwell, Oxford. pp. 61–93.
- Ikeda, T., Holtman, K., Kadla, J.F., Chang, H.-m., Jameel, H. (2001) Studies on the effect of ball milling on lignin structure using a modified DFRC method. *J. Agric. Food Chem.* 50:129–135.
- Kim, J.S., Awano, T., Yoshinaga, A., Takabe, K. (2010) Immunolocalization of  $\beta$ -1-4-galactan and its relationship with lignin distribution in developing compression wood of *Cryptomeria japonica*. *Planta* 232:109–119.
- Kleen, M., Gellerstedt, G. (1995) Influence of inorganic species on the formation of polysaccharide and lignin degradation products in the analytical pyrolysis of pulps. *J. Anal. Appl. Pyrolysis*. 35:15–41.
- Lommen, A. (2009) MetAlign: interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data preprocessing. *Anal. Chem.* 81:3079–3086.
- Mast, S.W., Donaldson, L.A., Torr, K., Phillips, L., Flint, H., West, M., Strabala, T.J., Wagner, A. (2009) Exploring the ultrastructural localization and biosynthesis of  $\beta$ (1,4)-galactan in *Pinus radiata* compression wood. *Plant Physiol.* 150:573–583.
- Minor, J.L. (1982) Chemical linkage of pine polysaccharides to lignin. *J. Wood Chem. Technol.* 2:1–16.
- Mohan, D., Pittman, C.U., Steele, P.H. (2006) Pyrolysis of wood/biomass for bio-oil: a critical review. *Energ. Fuel*. 20:848–889.
- Moldoveanu, S.C. *Analytical pyrolysis of natural organic polymers*. Elsevier Science, Amsterdam, 1998.
- Möller, R., McDonald, A., Walter, C., Harris, P.J. (2003) Cell differentiation, secondary cell-wall formation and transformation of callus tissue of *Pinus radiata* D. Don. *Planta* 217:736–747.
- Mukoyoshi, S., Azuma, J., Koshijima, T. (1981) Lignin-carbohydrate complexes from compression wood of *Pinus densiflora* Sieb. et. Zucc. *Holzforchung* 35:233–240.
- Nanayakkara, B. (2007) Chemical characterisation of compression wood in plantation grown *Pinus radiata*. Doctoral thesis, The University of Waikato.
- Nanayakkara, B., Manley-Harris, M., Suckling, I.D., Donaldson, L.A. (2009) Quantitative chemical indicators to assess the gradation of compression wood. *Holzforchung* 63:431–439.
- National Institute of Standards and Technology (2002) NIST/EPA/NIH Mass Spectral Database.
- Pan, W.-P., Richards, G.N. (1989) Influence of metal ions on volatile products of pyrolysis of wood. *J. Anal. Appl. Pyrolysis* 16:117–126.
- Ponder, G.R., Qiu, H.-X., Richards, G.N. (1990) Pyrolytic conversion of biomass to anhydrosugars. *Appl. Biochem. Biotechnol.* 24/25:41–47.
- Pouwels, A.D., Eijkel, G.B., Boon, J.J. (1989) Curie-point pyrolysis-capillary gas chromatography-high-resolution mass spectrometry of microcrystalline cellulose. *J. Anal. Appl. Pyrolysis* 14:237–280.
- R Development Core Team (2011) R: A language and environment for statistical computing. from <http://www.R-project.org>.
- Ralph, J., Hatfield, R.D. (1991) Pyrolysis-GC-MS characterization of forage materials. *J. Agric. Food Chem.* 39:1426–1437.
- Renard, C.M.G.C., Crépeau, M.-J., Thibault, J.-F. (1995) Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins. *Carbohydr. Res.* 275:155–165.
- Rencoret, J., Gutiérrez, A., Nieto, L., Jiménez-Barbero, J., Faulds, C.B., Kim, H., Ralph, J., Martínez, Á.T., del Río, J.C. (2011) Lignin composition and structure in young versus adult *Eucalyptus globulus* plants. *Plant Physiol.* 155:667–682.
- Sánchez, M., Pena, M.J., Revilla, G., Zarra, I. (1996) Changes in dehydrodiferulic acids and peroxidase activity against ferulic acid associated with cell walls during growth of *Pinus pinaster* hypocotyl. *Plant Physiol.* 111:941–946.
- Scurfield, G. (1973) Reaction wood: its structure and function. *Science* 179:647–655.
- Shafizadeh, F., Fu, Y.L. (1973) Pyrolysis of cellulose. *Carbohydr. Res.* 29:113–122.
- Syverud, K., Leirset, I., Vaaler, D. (2003) Characterization of carbohydrates in chemical pulps by pyrolysis gas chromatography/mass spectrometry. *J. Anal. Appl. Pyrolysis* 67:381–391.
- Terashima, N., Fukushima, K. (1988) Heterogeneity in formation of lignin – XI: An autoradiographic study of the heterogeneous formation and structure of pine lignin. *Wood Sci. Technol.* 22:259–270.
- Timell, T.E. *Compression Wood in Gymnosperms*. Springer Verlag, Berlin, 1986.
- Tokareva, E.N., Pranovich, A.V., Fardim, P., Daniel, G., Holmbom, B. (2007) Analysis of wood tissues by time-of-flight secondary ion mass spectrometry. *Holzforchung* 61:647–655.
- Torri, C., Adamiano, A., Fabbri, D., Lindfors, C., Monti, A., Oasmaa, A. (2010) Comparative analysis of pyrolysate from herbaceous and woody energy crops by Py-GC with atomic emission and mass spectrometric detection. *J. Anal. Appl. Pyrolysis* 88:175–180.
- Westermarck, U. (1985) The occurrence of *p*-hydroxyphenylpropane units in the middle-lamella lignin of spruce (*Picea abies*). *Wood Sci. Technol.* 19:223–232.
- Whistler, R.L., Chen, C.-C. (1991) Hemicellulose. In: *Wood Structure and Composition*. Eds. M. Lewin, I.S. Goldstein. Marcel Dekker, New York. pp. 287–319.
- Whiting, P., Goring, D. (1982) Chemical characterization of tissue fractions from the middle lamella and secondary wall of black spruce tracheids. *Wood Sci. Technol.* 16:261–267.
- Yeh, T.-F., Braun, J.L., Goldfarb, B., Chang, H., Kadla, J.F. (2006) Morphological and chemical variations between juvenile wood, mature wood, and compression wood of loblolly pine (*Pinus taeda* L.). *Holzforchung* 60:1–8.
- Yeh, T.-F., Goldfarb, B., Chang, H., Peszlen, I., Braun, J.L., Kadla, J.F. (2005) Comparison of morphological and chemical properties between juvenile wood and compression wood of loblolly pine. *Holzforchung* 59:669–674.
- Zykwinska, A., Thibault, J.-F., Ralet, M.-C. (2007) Organization of pectic arabinan and galactan side chains in association with cellulose microfibrils in primary cell walls and related models envisaged. *J. Exp. Bot.* 58:1795–1802.
- Zykwinska, A.W., Ralet, M.-C.J., Garnier, C.D., Thibault, J.-F.J. (2005) Evidence for in vitro binding of pectin side chains to cellulose. *Plant Physiol.* 139:397–407.